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E.1 NRDA Sampling Team

The composition and size of the team involved in data collection and analysis during the Preassessment Phase will vary according to the complexity of the oil spill incident. For incidents where a moderate to large effort is required for preassessment activities, the following specific core team members are usually needed, either on-scene or as support staff to the field team:

- Trustee Agency Coordinator (from all of the trustees)
- Natural Resource Biologist (for each of the major resources/habitats affected by the incident)
- Environmental (Petroleum) Chemist
- Natural Resource Economist
- Restoration Expert
- Administrative Specialist
- QA Specialist
- Data Manager/Sample Custodian
- Statistician
- Natural Resource Attorney

The responsibilities of each core team member are discussed below.

Trustee Agency Coordinator: The trustee coordinator must be an agency employee who has the authority to represent the trustees. Responsibilities include:

- Attend trustee meetings;
- Represent trustee responsibilities at meetings;
- Determine the potential for effects to trust resources and services;
- Organize trustee meetings and involvement when lacking;
- Evaluate the adequacy of current NRDA activities; and
- Recommend activation of additional team members.

Natural Resource Biologist: Responsibilities include:

- Develop familiarity with the local habitats and biological resources;
- Determine the natural resources potentially at risk;
- Assist in developing consensus among trustees on natural resources and services at risk and objectives of data collection plans;
- Design and implement necessary and relevant data collection activities and implement sampling and testing programs;
- Identify data gaps in current NRDA activities; and
- Determine need for specialized or local experts and guide work of experts along NRDA requirements.

Environmental (Petroleum) Chemist: Responsibilities include:

- Evaluate the chemical fate and effects of the discharged substance and use this information in the development of appropriate data collection plans;

- Review and update plans for collection and processing of chemical samples; and

- Evaluate or specify the proposed analytical protocols, reviewing the protocols of the selected laboratory to determine their ability to meet the data quality requirements.

Natural Resource Economist: Responsibilities include:

- Assist in developing consensus among trustees on resource services at risk and objective of related data collection plans;

- Provide expertise on the breadth of natural resource services;

- Conduct scaling of various restoration actions;

- Conduct the other economic components of the damage assessment; and

- Work closely with the core team members to determine the appropriate assessment method and ensure that the assessment is addressing the issues of greatest importance to the damage assessment.

Restoration Expert: Responsibilities include:

- Collect information on restoration alternatives;

- Identify the types of studies needed to support selection of restoration alternatives;

- Initiate the development of the restoration plan; and

- Develop monitoring plans and project activities related to restoration planning and implementation.

Administrative Specialist: The information specialist is an incident-specific, on-site role rather than a member of the planning team. Responsibilities include:

- Collect reports, write minutes of meetings, and file technical and response data in an organized manner with full documentation;

- Make sure that all NRDA meetings have prepared minutes;

- Prepare a Daily Activities Log for the record; and

- Process contracts and other financial needs to facilitate progress relative to the incident.

QA Specialist: Responsibilities include:

- Review/update QA protocols for all data collection activities; and

- Make sure that sampling activities follow QA protocols.

Data Manager/Sample Custodian: Although many of the tasks conducted by the data manager are part of QA, an on-site person should be assigned these operational responsibilities, which include:

- Develop a consistent database on NRDA sampling efforts (i.e., who, what, where);

- Track sample chain-of-custody forms;

- Review and approve data forms;

- Responsibility for receiving, properly storing, and shipping samples; and

- Prepare and deliver a complete file on the incident to the trustees, with an electronic index or reference list.

Statistician: Responsibilities include:

- Be familiar with all types of environmental data analyses including parametric and nonparametric statistics and multi-variate techniques;
- Have a practical understanding of field sampling methods and issues;
- Design statistically sound sampling strategies; and
- Monitor implementation of the sampling strategy and suggest changes when needed.

Natural Resource Attorney: Responsibilities include:

- Provide analysis of legal considerations in the NRDA;
- Play a key role in representing trustees to the RPs;
- Provide analyses of past NRDA cases;
- Draft agreements among the trustees and between the trustees and RPs; and
- Provide legal advice to the trustees throughout the NRDA process.

Depending on the conditions of the incident, specialized technical experts may be needed on the NRDA team at the very beginning. These technical experts can contribute to the early effort of the team remotely by telecommunications and provide recommendations (e.g., methodologies, etc.) or go on-scene and direct the activities themselves.

One person can take on multiple roles, depending on the scale and phase of the NRDA response. Initially, at least two team members are needed to go on-scene to evaluate the incident conditions, collect the information needed to determine the level of response, and prepare a preliminary sampling strategy. One of these members should include the information specialist. Other team members and experts can be mobilized as needed.

E.2 On-Scene Assessment

In order to conduct preassessment activities, for most oil spill incidents, the trustees may need to go on-scene to collect pertinent data. The presence of the trustees is necessary because so much information is generated and exchanged verbally on-scene among responders or is incompletely documented.

E.2.a Data Collection and Analysis Activities

Once the trustees arrive on-scene, the first objective is to survey and document the nature and extent of injury to natural resources and services, and identify those resources and services that may be affected in the future. There are several tasks that need to be completed as part of preassessment. These tasks include:

- Collection and assessment of readily available information on the type, amount, characteristics, location, and predicted trajectory of the discharged oil;

- Development of an abbreviated data collection plan for the NRDA response;

- On-scene assessments of the incident, through reconnaissance surveys and on-site inspections of areas that may be affected;

- Development and implementation of a more detailed data collection and analysis plan for early sampling to determine the potential exposure and injuries of natural resources and services; and

- Documentation and reporting requirements.

Exhibit E.1 includes a checklist for NRDA trustees to refer to during the first few days of the on-scene data collection activities. This checklist is not all-inclusive, but should be used as a guide for the types of issues addressed. Further, NOAA has prepared an Emergency Procedures Manual (NOAA, 1994), which provides guidance to NRDA teams on early sampling activities during oil spill incidents.

- Obtain a briefing on the status of the incident from the OSC or SSC at the Command Post. Get updated information on the incident, potential discharge amount, response actions, trajectory of the discharge, and weather conditions that might affect discharge conditions, etc
- Evaluate the site safety issues. Obtain a copy of the site safety plan, if available.
- Establish a filing system and begin filing all documents.
- Obtain copies of local charts and topographic maps of the incident area. Make photocopies of the incident site for recording information during overflights and ground surveys.
- Obtain copies of any and all reports (e.g., pollution reports, Hotline, operational summaries), maps, and trajectories on the discharge from the Command Post, newspaper articles, press releases, response plans, etc.
- Obtain a briefing on the natural resources and services at risk, reports on effects to-date, and names of contacts for the resource agencies involved in the response. Identify all response activities by resources agencies.
- Get on an overflight as soon as possible. Bring maps or charts, camera and/or videocamera, overflight checklist, and necessary safety equipment. Take sufficient photographs; keep a detailed photolog.
- Ship in supplies and response kits that may be needed.
- Check contents of kits and determine need for supplemental equipment and supplies (e.g., film, batteries, videotapes, more sampling jars, cleaning and preservation chemicals, etc.).
- Get tide schedules.
- Develop an initial data collection plan, including samples from the source.
- Determine schedule of daily meetings so that the appropriate people attend.
- Establish a NRDA work area. Rent cellular phones. Distribute names/phone contacts for the team.
- Determine need for contractor support.
- Develop an early data collection plan to collect data on oil effects to natural resources and services, making sure to coordinate with response activities. Identify baseline and reference/control conditions.
- Form NRDA teams to implement the sampling plans. Make sure everyone on the teams has appropriate safety gear and training.
- Make sure that maps showing the distribution of the discharge over the area and time are generated in a systematic manner.
- Make sure that there are written QA protocols for data collection and that these protocols are understood by all NRDA team members.
- Distribute copies of reports on natural resources and services at risk and sampling plans to home offices.
- Evaluate the need for experts. Identify potential experts as needed.
- Brief SSC and other operational responders on NRDA activities.
- Generate lists of names, phone contacts, and activities for all persons involved with data collection.
- Review OPA Fund procedures.
- Consider emergency restoration actions.
- Implement documentation procedures by distributing forms for field observations, chain-of-custody, photo logs, daily activities reports, etc.
- Coordinate with biological rescue and rehabilitation groups to make sure that detailed documentation of number, species, and fate of animals is kept.
- Keep track of shoreline, waterway closures, and seafood safety procedures.
- Consider damage assessment options.

Exhibit E.1 Checklist of preassessment activities for NRDA trustees.

The trustees should develop an early study plan for collecting necessary and relevant data on the natural resources and services that may be affected. This plan will be used in the Preassessment Phase to select the type of damage assessment to conduct. The plan should clearly state: the objectives of the study; which natural resources and services are to be studied; the type of samples that are to be collected; the frequency of sampling; and the type of analysis anticipated. Each study plan will be unique for the current incident conditions and must have the flexibility to incorporate new objectives as the on-going incident conditions change. The plan should be written and distributed to the other trustees for their approval and participation.

E.2.b Aerial Reconnaissance

The initial identification of areas potentially affected is usually conducted through aerial reconnaissance. During reconnaissance, documentation of the extent of the incident is very important for establishing monitoring stations and baseline conditions. This information is not always routinely collected by the response team at the scale, frequency, or detail required for later analysis under NRDA.

Aerial surveys allow an extremely rapid assessment of the entire incident site and are especially useful in determining the relationship between local geomorphology and contaminant distribution as well as the areal extent of effects (e.g., heavy accumulations of oil in east-facing pocket beaches, oil sheens in backwater lakes along a river). Inflight observations are recorded both orally on audio tape and photographically with a hand-held 35 mm camera or a videocamera preferably both. The best altitude for observations is between 500 and 1,500 feet. Either a helicopter or fixed-wing (i.e., high-wing) aircraft may be used.

Primarily, the overflights are conducted to determine the extent of contamination or habitats that may be contaminated, and observe biological resources and services highly susceptible to future effects.

Some important guidelines for conducting a safe and productive overflight are outlined below:

- Learn the communications protocol in the aircraft (i.e., when not to talk, who is directing the pilot, and how to communicate your objectives and plans);

- Go over the flight plan with the pilot. Have a map of the flight plan sketched out, if possible;

- Always have your own maps and know the area surveyed;

- Keep track of your location and elevation;

- Plot your flight path on your map, noting the time every 30 minutes or when you change direction;

- Make detailed observations of affected natural resources or the proximity of natural resources (e.g., biological, habitat, etc.) and services (i.e., ecological and public) relative to the incident;

- When mapping oil onshore, carefully locate yourself with land features. Use detailed respirators for contaminant type, coverage, dimensions, behavior (e.g., sheening, refloating, or lack thereof). Draw directly onto the map. Note the presence of response workers, locations and estimates of number of biota by species, and observations on position of the oil relative to natural resources and services. Use a pre-determined code for describing oil type (e.g., mousse, black oil, rainbow sheen, etc.) and coverage. Exhibit E.2 contains recommended terminology for oil descriptions and terms;

- Immediately after returning to the Command Post or base of operations, transfer your observations to a worksheet and a clean copy of a map while the information is still fresh; and

- If photographs are taken, check your photo log and make sure it is completed. Identify the location of all photos on the map as they are taken to create an accurate record of the overflight.

E.2.c Ground Reconnaissance Studies

Once the overflight is complete, ground surveys are needed to groundtruth the aerial observations and collect samples. These ground surveys are conducted with the realization that future monitoring studies may be established in some of these areas, so detailed records should be made of all observations.

Shoreline Slope

Low	Less than 30 degrees
Medium	Between 31 and 60 degrees
High	Between 61 and 90 degrees
Vertical	Vertical or near vertical

Oil Category Width

W	Wide	> 6 m wide
M	Medium	>3m to <6m
N	Narrow	> 0.5 m to < 3 m
V	Very Narrow	≤ 0.5 m

Oil Distribution

C	Continuous	91 - 100%
B	Broken	51 - 90%
P	Patchy	11 - 50%
S	Sporadic	1 - 10%
T	Trace	<1%

Shoreline Zone

SU	Supratidal (above normal spring high tide levels)
UI	Upper Intertidal
M	Middle Intertidal
LI	Lower Intertidal

Sediment Type

R	Bedrock outcrops
B	Boulder (>256 mm in diameter)
C	Cobble (64-256 mm)
P	Pebble (4-64 mm)
G	Granule (2-4 mm)
S	Sand (0.06-2 mm)
M	Mud (silt and clay, < 0.06 mm)
RR	Riprap (man-made permeable rubble)
SW	Seawalls (impermeable)

Surface Oiling Descriptors - Thickness

PO	Pooled Oil (fresh oil or mousse > 1 cm thick)
CV	Cover (oil or mousse from >0.1 cm to <1 cm on any surface)
CT	Coat (visible oil <0.1 cm, which can be scrapped off with fingernail)
ST	Stain (visible oil, which cannot be scraped off with fingernail)
FL	Film (transparent or iridescent sheen or oily film)

Surface Oiling Descriptors - Type

FR	Fresh Oil (unweathered, liquid oil)
MS	Mousse (emulsified oil occurring over broad areas)
TB	Tarballs (discrete accumulations of oil <10 cm in diameter)
PT	Patties (discrete accumulations of oil >10 cm in diameter)
TC	Tar (highly weathered oil, of tarry, nearly solid consistency)
SR	Surface Oil Residue (non-cohesive, heavily oiled surface sediments, characterized as soft, incipient asphalt pavements)
AP	Asphalt Pavements (cohesive, heavily oiled surface sediments)
NO	No Oil
DB	Debris; logs, vegetation, rubbish, garbage, response items such as booms, etc.

Subsurface Oiling Descriptors

SAP	Subsurface asphalt pavement (cohesive)
OP	Oil-Filled Pores (pore spaces are completely filled with oil, to the extent that the oil flows out of the sediments when disturbed). May also consist of weathered oil such as a buried lens of asphalt pavement
PP	Partially Filled Pores (pore spaces partially filled with oil, but the oil does not flow out of the sediments when disturbed)
OR	Oil Residue (sediments are visibly oiled with black/brown coat or cover on the clasts, but little or no accumulation of oil within the pore spaces)
OF	Oil Film (sediments are lightly oiled with an oil film, or stain on the clasts)
TR	Trace (discontinuous film or spots of oil, or an odor or tackiness)
NO	No Oil (no evidence of any type of oil)

Sheen Color

B	Brown
R	Rainbow
S	Silver
N	None

Exhibit E.2 Shoreline oil terminology/codes.

When physically visiting exposed sites or potentially affected areas, the trustees should record the extent of effects (i.e., discharge distribution, percent coverage, zone of coverage, etc.), create visual records of the sites (i.e., photographs, video records, field sketches, oral descriptions on tape, etc.), collect samples as needed, and identify areas that may serve as likely baseline and reference/control sites. Systematic documentation of observations is extremely important. Exhibit E.2 includes recommended terminology and codes for use in shoreline surveys. Detailed guidance can be found in the Emergency Procedures Manual (NOAA, 1994), which provides guidance to NRDA teams on early sampling activities during incidents. Methods for systematic shoreline surveys are also described in Owens (1991) and Debusschere et al. (1992).

Hand-held Global Positioning System (GPS) units make sample location easy to determine and record. GPS is a satellite positional and navigation network, which allows determination of a position fix within tens of meters.

E.3 Chemical Sampling

E.3.a Objectives

Stating a clear objective often seems so obvious that it is not explicitly mentioned by investigators considering sampling projects. However, it is frequently the case that objectives are not well formulated, leading to inconclusive results. With clearly stated objectives, the trustees can be confident in the design and implementation of data collection and analysis plans. During preassessment activities, chemical samples are collected primarily to answer the following basic questions:

- Is this oil the same as the oil that was discharged?
- What is the concentration of the oil in the media being sampled?
- What is the composition of the oil in the media being sampled?

The quality of the results obtained from the sample analysis is directly related to:

- Collecting representative samples;
- Using appropriate sampling techniques; and
- Properly preserving the samples until they are analyzed.

E.3.b Chemical Sampling Methods

E.3.b.1 Environmental Samples for Fingerprint Analysis

Samples for fingerprint analysis are collected to answer the question "Is this oil the same as the oil that was discharged?" Consequently, such samples do not have to be quantified or related to a measured amount of sample. Fingerprint samples are grab samples, not composites. They are compared with a "source" sample, preferably one carefully collected directly from the original container (i.e., vessel, pipeline, tank) and not from floating slicks or stranded oil. The following techniques are used to collect thin sheens -- for offshore, a special sheen sampler composed of teflon strips on a line is pulled through the sheens, -- for nearshore, teflon filter papers are used to pick up small sheens (Henry, 1993). In all cases, special care is taken to prevent contamination with sheens from the exhaust of boat motors or washing off the sides of the sampling platform. Samples for fingerprinting can also include tarballs, oiled sediments, oiled animal parts (i.e., preferably fur/feathers), and oiled vegetation. Biological samples are collected in glass jars or aluminum foil and kept frozen until analysis. Sample containers, sample volumes, and holding times are listed in Exhibit E.3. All lids should be aluminum- or teflon-lined, not plastic.

E.3.b.2 Collection of Water Samples

Water samples are very difficult to collect where surface oil slicks are present. When sampling in areas covered by a surface slick, extreme care is needed to "knock" the surface slick a side so that the sampling device can be lowered into the water without becoming contaminated. Water samples should be collected directly in the sample container, since oil droplets readily adhere to the inside of most samplers. For shallow water samplers, it is necessary to wade into the water or lean over from a platform to collect the sample. The container is opened, allowed to fill, and closed at the desired water depth. Deeper water samples have to be collected by lowering the sampling container to the desired depth and remotely opening and closing the container at that depth. Under most conditions, concentrations in the water will be very low and the potential for contamination is high. Additionally, surface slicks and the water-accommodated fraction track differently. Therefore, the presence of oil on the water surface is not always an appropriate criterian for collection of water-column samples.

Depending on the analytical method used, minimum sample volumes range from one liter to one gallon. With replication, the volume of water shipped to the laboratory can be significant. Ideally, water samples should be stored no longer than eight hours, although, if they are kept cold (4°C) and dark, water samples may be stored for as long as five days. Background samples should be collected well away from any oiled areas, being careful not to sample along the vessel path or near any boat or aircraft engines, to reduce the chance of contamination.

E.3.b.3 Collection of Sediment Samples

Sediment samples are collected from two general settings; surface sediments (i.e., terrestrial soils and intertidal sediments), which are readily visible and collected by hand and subtidal sediments, which are usually sampled with a remote sampling device. Intertidal surface sediments and soils are relatively easy to sample since variability in the sediment type and degree of contamination can be readily discerned and described. Two of the most difficult issues in sediment sampling at oil discharges are:

- Collection of a representative sample where there is extreme variability in the amount of oil. On an oiled marsh, the contamination will range from oil pooled on the surface to patches adjacent to visibly clean sediments. This problem is even more difficult where the sediments are coarse (i.e., in the range of pebbles to cobbles) and where the natural variability is compounded by the size of the material compared to the sampling container. One approach is to composite subsamples, but this approach does not facilitate statistical analysis of the variability at a site.

- Prevention of cross contamination when moving between heavily contaminated sites and clean sites. Keeping sampling equipment and supplies clean between sites at an oil discharge is very difficult. The only guarantee against cross contamination is to use new, clean utensils at every sampling site. On-site cleaning of utensils or corers is not recommended. Blanks are extremely important to collect, particularly at the cleaner sites.

Detailed descriptions and photographs of the samples and site are needed. Photoscales are always used when photographing sediment sampling sites. Documentation includes both overview and close-up photographs. To determine oil penetration with depth, trenches are dug, described, and photographed. Grain-size estimators (i.e., field guides that visually show grain-size classes) are used to describe sediment textural parameters. Standard oil descriptors are used to characterize the type and degree of contamination. Sampling site locations are shown on detailed field sketches or maps.

Exhibit E.3 Sample requirements for discharges of oil.

Oil Analysis	Quantity	Storage	Other Conditions
Fingerprinting slick or tarball samples.	> 100 ml	Precleaned glass jars or aluminum foil	Kept cold or frozen until delivered to the lab.
Fingerprinting source samples	~ 1 gallon	Precleaned glass containers	Kept cold or frozen until delivered to the lab.
Weathering source samples	~ 1 liter	Precleaned glass jars	Kept cold or frozen until delivered to the lab.
Water Samples	~ 1 gallon	Super-clean glass containers	Kept cold and dark for less than 8 hours. Preservation of sample may be done — check with analytical lab.
Sediment Samples, Fingerprinting	100 grams	Precleaned glass jars or aluminum foil	Extreme care should be taken to prevent sample contamination. Kept cold or frozen until delivered to the lab.
Sediment Samples, Total Petroleum Hydrocarbons	100 grams	Precleaned glass jars or aluminum foil	Composite subsamples, 10-15 samples from site. Kept cold or frozen until delivered to the lab.
Sediment Samples, Grain Size	50-100 grams	Ziploc or whirl-pak bags	No special conditions required.

Subtidal or bottom sediment samples pose a different set of problems mainly because they require use of sampling devices deployed from a boat or diver-held corers. Samplers that take box cores with minimal disturbance of the surface are the preferred device because most of the contamination in the first few weeks is likely to be on the sediment surface. Box corers also allow ready collection of replicates or composites at a site. Cleaning of the sampling device is a second problem, especially when the sediment contamination levels are high. Soap and water, scrub brushes, large volumes of clean water, and solvent rinses are needed to clean the sampling device between stations. Collection of the waste solvent for disposal must also be addressed.
It is important to record water depth, time of collection (i.e., so depth can be corrected to mean sea level in tidal waters), sediment description, visible oiling conditions, depth of bioturbation, presence of oxidized and reduced zones, and presence and relative density of invertebrates. The sediment surface should be photographed prior to collection from the corer.

Samples of approximately 100-200 grams (i.e., approximately one cup) are usually sufficient. The data collection plan should specify the sample intervals and depths. All samples should be numbered uniquely. It is important to have preassigned numbering series for samples when multiple teams are in the field collecting samples. Samples are collected with clean metal utensils or wooden tongue depressors and placed in precleaned glass jars with teflon-lined caps.

E.4 Toxicity Testing

E.4.a Objectives

Toxicity tests are used to determine whether discharged oil and its degradation by-products have measurable effects on the biota potentially exposed during an incident. When combined with field surveys documenting direct effects in the field, toxicity data establish the link between the incident and ecological responses (Parkhurst et al., 1989). The objectives of toxicity tests are to correlate an adverse ecological response with exposure to the discharged oil and determine the concentrations at which response occurs for the species of concern. Toxicity test results can be used in preassessment as a screening tool to indicate potential biological effects and whether further bioassessment studies are needed.

There are two approaches to determine toxicity of a substance, the *toxicity-based approach,* which directly measures the biological effect resulting from exposure to the contaminated pathway from the incident site, and the *chemistry-based approach* which uses chemical analyses and laboratory-generated criteria (e.g., LC50 values) to estimate toxicity (Parkhurst et al., 1989). Both of these approaches are complementary and may be used for quantifying toxicity of water and sediments contaminated by a discharge of oil.

E.4.b Methods

The following toxicity tests are examples of those suitable for use in preassessment. There are many more that may be appropriate for specific discharge conditions. While the most commonly measured endpoint is death or immobility of the organisms, other endpoints frequently measured include changes in reproduction, growth, development, and behavior.

The toxicity tests listed in Exhibit E.4 are representative of commonly used types of standardized tests As long as accepted protocols are followed, there is the potential for establishing local or regional test protocols with endemic species or species of concern.

E.5 Biomarkers

Biomarkers are direct biological measurements used to quantify the degree of exposure or response to the contaminant (Hunsaker and Carpenter, 1990). More specifically, biomarkers are "...biochemical, physiological, or histological indicators of either exposure to, or effects of, xenobiotic chemicals at the suborganismal or organismal level" (Huggett et al., 1992). There are two types of biological indicators, exposure and response indicators. Exposure indicators establish that organisms were subjected to a potentially deleterious stressor and quantify the extent of that exposure. However, exposure indicators cannot be used to detect deleterious effects. In contrast, response indicators demonstrate that deleterious effects are occurring, although often it is difficult to link the cause of the effect to exposure to the discharged oil. Thus, in most instances, both response and exposure indicators are needed to establish that effects are occurring and link the cause of those effects to oil exposure. Biomarker tests currently applied to specific groups of organisms are listed below (MacDonald et al., 1992):

Mollusks	Mixed function oxygenase (MFO) enzymes Binding proteins Neoplasms Lesions and other histopathological disorders Growth reduction
Fish	Mixed function oxygenase (MFO) enzymes Binding proteins Lesions and other histopathological disorders Deoxyribonucleic acid (DNA) adducts Skeletal defects Polyaromatic hydrocarbon (PAH) metabolites in bile Reproductive measures
Birds/Marine Mammals	Mixed function oxygenase (MFO) enzymes Reproductive measures Lesions and other histopathological disorders

Exhibit E.4 Freshwater, and marine and estuarine toxicity tests.

Test	Endpoint	Duration	Reference
Freshwater *Water Column* Acute Toxicity Tests LC_{50}, EC_{50}, or IC_{50} Fish - Rainbow trout (*Oncorhynchus mykiss*) - Coho salmon (*O. kisutch*) - Brook trout (*Salvelinus fontinalis*) - Fathead minnow (*Pimephales promelas*) - Bluegill (*Lepomis macrochirus*) - Goldfish (*Carassius auratus*) - Channel catfish (*Ictalurus punctatus*) - Green sunfish (*Lepomis cyanellus*)	survival	2 to 8 days	APHA, 1989; ASTM, 1992 USEPA, 1985 ASTM, 1992 ASTM, 1992 ASTM, 1992; USEPA, 1985 ASTM, 1992; USEPA, 1985 ASTM, 1992 ASTM, 1992 ASTM, 1992
Invertebrates - Daphnids (*Daphnia* sp.) - Water flea (*Ceriodaphnia dubia*) - Amphipods (*Gammarus* sp.) - Crayfish (*Orconectes* sp., *Cambarus* sp., *Procambrus* sp., *Pacifastacus leniusculus*) - Mayflies (*Hexagenia* sp.) - Midges (*Chironomus* sp.) - Snails (*Physa* sp. and *Amnicola limosa*)	immobilization and/or survival	2 to 8 days	ASTM, 1992; USEPA, 1985

APHA = American Public Health Association
ASTM = American Society for Testing and Materials
PSEP = Puget Sound Estuary Program
USEPA = U.S. Environmental Protection Agency

Freshwater (continued) Chronic Toxicity Tests Early Life Stage Toxicity Tests Fish - Salmonids - Fathead minnow - White sucker (*Catostomus commersoni*) - Channel catfish - Bluegill - Striped bass (*Morone saxatilis*)	 survival/ abnormal development survival/growth survival/growth survival/growth survival/growth survival/growth	 30 days post swim-up 32 days 32 days 32 days 32 days 45 days	 ASTM, 1992
Life-Cycle Tests Invertebrates - *Ceriodaphnia dubia* partial-life cycle test - *Daphnia magna* life-cycle test	 survival/ reproduction	 7 days 21 days	 USEPA, 1989 USEPA, 1989
Algal Growth Test - *Selenastrum capricomumtum*	 growth	 96 hours	 USEPA, 1985
Sediment (*Sediment Elutriate*) Invertebrates - Amphipod (*Hyalella azteca*) - Midge (*Chironomus* sp.)	 survival/growth survival/growth	 10 days 10 days	 ASTM, 1992

Marine and Estuarine *Water Column* Acute Toxicity Tests LC_{50}, EC_{50}, or IC_{50} Fish - Sheepshead minnow (*Cyprinodon variegatus*) - Mummichog (*Fundulus heteroclitus*) - Longnose killfish (*Fundulus sirnilis*) - Silversides (*Menidia* sp.) - Threespine stickleback (*Gasterosteus aculeatus*) - Pinfish (*Lagodon rhomboides*) - Spot (*Leiostomus xanthurus*) - Shiner perch (*Cymatogaster aggregata*) - Tidepool sculpin (*Oligocottus maculosus*) - Sanddab (*Citharichthys stigmaeus*) - Flounder (*Paralichthys dentatus, P. lethostigma*) - Starry flounder (*Platichthys stellatus*) - English sole (*Parophrys vetulus*) - Herring (*Clupea harengus*)	survival	2 to 8 days	ASTM, 1992

Invertebrates - Copepods (*Acartia* sp.) - Shrimp (*Penaeus* sp., *Pandalus* sp.) - Grass shrimp (*Palaemonetes* sp.) - Sand shrimp (*Crangon septemspinosa*) - Mysid (*Mysidopsis* sp.) - Blue crab (*Callinectes sapidus*) - Shore crab (*Hemigrapsus* sp., *Pachygrapsus* sp.) - Green crab (*Carcinus meanas*) - Fiddler crab (*Uca* sp.) - Oyster (*Crassostrea* sp.) - Blue mussel (*Mytilus edulis*) - Hard clam (*Mercenaria mercenaria*) - Polychaete (*Capitella capitata*)	immobilization and/or survival	2 to 8 days	ASTM, 1992
<u>Chronic Toxicity Tests</u> <u>Early Life-Stage Toxicity Tests</u> Fish - Gulf toadfish (*Opsanus beta*) - Sheepshead minnow - Atlantic silversides (*Menidia menidia*) - Inland silversides larval test (*M. berylina*)	survival/growth	42 days 28 days 28 days 7 days	ASTM, 1992
Invertebrates Echinoderm sperm cell fertilization test - Sea urchin (*Strongylocentrotus droebachienses*) - Sand dollar (*Dendraster excentricus*)	fertilization	200 minutes	Dinnel et al., 1987

Bacteria - *Photobaterium phosphoreum* (Microtox®)	biolumin-escence	15 minutes	PSEP, 1991
Sediments			
Fish Anaphase abberation test (rainbow trout gonad cells)	genotoxicity/ cytotoxicity	48 hours	PSEP, 1991
Invertebrates Amphipods - *Phepoxynius abronius* - *Eohaustorius estuarius* - *Ampelisca abdita* - *Grandidierella japonica*	survival/ avoidance	10 days	ASTM, 1992
Polychaete (*Neanthes* sp.)	survival/growth	20 days	PSEP, 1991
Bacteria - *Photobaterium phosphoreum* (Microtox®)	biolumin-escence	15 minutes	PSEP, 1991

E.5.a Exposure Indicators

The most appropriate biochemical endpoints to measure exposure to oil and its by-products include bioaccumulation in tissues and presence of hydrocarbon metabolites in bile. Bioaccumulation in tissues is a direct measure of exposure and applicable to oil discharge conditions. Petroleum hydrocarbons are highly lipophilic and known to have high bioconcentration factors for many organisms, particularly shellfish (Scott et al., 1984). There are numerous studies on bioconcentration factors, uptake, and depuration-rate kinetics for different species and biological organs.

One commonly used technique for bioaccumulation studies is the deployment of mussels collected from resident natural populations in the affected area to measure both bioavailability and degree of exposure (Salazar, 1992). Mussel collection procedures are relatively easy and chemical analyses of tissues are standardized (NOAA, 1989). Salazar (1992) provides the following guidelines for using mussels in support of natural resource damage assessment studies:

- To discriminate differences of exposure and response among assessment sites, a 12-week exposure period is recommended;

- Adults appear more sensitive and have lower survival rates than juvenile mussels;

- Size sorting of individual organisms is important because juvenile mussels grow faster and appear to accumulate higher concentrations of contaminants in their tissues than adults;

- To increase survival rates during deployment, large clusters should be separated by hand, not machine; and

- If determination of sublethal effects, such as growth rates, is an objective, it is important to have adequate replication and measurement of each live animal at the beginning and end of the exposure.

Resident mussel populations can be used for monitoring if the area affected by the discharge includes stations with an historical record of levels of contaminants as suggested by Brooks et al. (1991).

A second category of exposure indicators are those that indirectly measure exposure by detecting a biochemical response to the exposure. This approach is used, where the hydrocarbons do not readily bioaccumulate, such as for fish which rapidly metabolize the oil (Varanasi et al., 1989); when direct measurement of the contaminant is difficult, or when the contaminant was depurated from the organism. Appropriate measurements for oil discharge studies include metabolites in the bile of fish (Varanasi et al., 1989) and hepatic MFO enzyme activity in fish and molluscs (Payne et al., 1986; Collier and Varanasi, 1991). The measurement in fish of reactions catalyzed by the hepatic cytochrome P4501A may prove useful in monitoring oil discharge effects. However, much more work is required to translate exposure and sublethal stress, as detected by monitoring of biomarkers, into biological effects at the population, community, or ecosystem level.

E.5.b Response Indicators

Response indicators are used to demonstrate that effects occurred as a result of exposure to the discharged oil. Although work was completed on response indicators at various levels of ecological organization, from the individual to the community (Hellawell, 1986; Hunsaker and Carpenter, 1990), the most timely indicators are survival and histopathology at the individual organism level for preassessment data collection.

Reduction in survival is a biological response that integrates exposure to environmental concentrations of contaminants. It is one of the least sensitive endpoints because mortality is an all-or-nothing response. However, important information can be gained from monitoring survival of selected organisms, especially where toxicity responses are well documented. Histopathology, the study of tissue responses to injury or disease, can be an important tool during preassessment to determine whether the cause of death of an organism could be related to exposure to the oil discharge and as an early indication of biological effects resulting from sublethal exposure. Most research on histopathological biomarkers have focused on the liver of higher organisms such as fish, where neoplasms (i.e., cancerous tumors), areas of excessive growth, and specific degenerative or necrotic lesions are reported (Myers et al., 1990). Some of these responses are from long-term, chronic exposures. More appropriate during preassessment might be indicators of acute exposures, such as injuries to the gills, lesions on the skin, eyes, and lips of fish. Histopathological studies of dead birds and mammals are often used to determine the cause of death.

Most of the time, histopathological analyses are conducted by Federal or State laboratories, the agencies that manage the affected resource. These agencies have the expertise needed to properly collect organisms, prepare the tissue sections, and examine and interpret the data.

E.6 Ecological Sampling

The wide range of biological systems potentially affected by an incident makes it extremely difficult to identify specific ecological sampling protocols appropriate for preassessment activities. For a specific natural resource, experts knowledgable in quantitative field methods and data analysis should be consulted to assist in the design of data collection plans. The types of ecological sampling that may be conducted during preassessment include census surveys of populations, number of acutely affected organisms, number of dead organisms, various types of reproductive failure, and collection of samples for chemical and histopathological analysis.

Rapid bioassessment techniques are useful as quick, screening tools to determine if there is a need for more detailed, quantitative surveys. For example, the USEPA has published rapid bioassessment protocols for use in streams and rivers for benthic macroinvertebrates and fish (Plafkin et al., 1989). Both the USFWS and American Fisheries Society have published methods for investigation of fish kills that are appropriate for incidents in riverine and estuarine settings (Meyer and Barclay, 1990; American Fisheries Society, 1992). The American Society for Testing and Materials (1992) has published standard methods for many different types of ecological surveys. Wildlife surveys commonly use transect, quadrat, and trap counts to estimate the total population present within the study area (Anderson et al., 1976; Davis and Winstead, 1980). Assessments of intertidal communities are discussed by Gonor and Kemp (1978) and Moore and McLaughlin (1978). Many of the papers listed in Appendix C include field methods for bioassessments.

E.7 Data Collection on Recreational Participation

E.7.a Objectives

Recreation participation sampling is used to determine whether discharged oil and its degradation by-products have measurable effects on the quantity and/or quality of recreation activities. During preassessment activities at an incident, data on recreational participation are collected primarily to answer the following basic questions:

- Are there recreation activities in the incident location that might be affected?
- What are the likely effects of the incident on these recreational activities?
- Are there other locations where people may recreate instead of the incident area?

- What types of data have been previously collected for each activity at each site?

Categories of recreation activities to consider include (but are not limited to):

- Beach Recreation
- Fishing:
  - ♦ Shore/Jetty
  - ♦ Pier
  - ♦ Private Boat
  - ♦ Charter/Party Boat
- Boating
  - ♦ Motor boating
  - ♦ Sailing
- Nature Viewing:
  - ♦ Photography
  - ♦ Specimen Collecting
- Specialized Activities:
  - ♦ SCUBA Diving
  - ♦ Kayaking/Canoeing
  - ♦ Small water craft use
  - ♦ Wind surfing

E.7.b Methods

During preassessment activities, tasks should include the determination of official or *de facto* closures, identification of uses by activity and location for the affected and potentially affected areas, assessment of the availability of use level data, and initial evaluation of potential levels of injury.

E.7.b.1 Determination of Closures

Official or de facto closures of recreation sites should be determined. De facto closures include “non-official” closures due to restrictions on access to the site or limitations on activities at the site as a result of the incident and response to the incident. These closures may be imposed by local municipalities, USCG, or other official agencies as a result of health concerns, response activities, or other reasons. Determination of official closures should be made from those officials issuing the closure notice. Determination of *de facto* closures should be made from local officials and/or response personnel.

E.7.b.2 Determination of Locations of Recreational Activity

The location of activities, likely substitutes for potential injured (or closed) sites, and probable effect from the incident, can be determined through interviews with knowledgeable local officials, and/or current users. Possible interview questions should determine the types of recreation that occur at sites most likely affected by the incident, recreation patterns (i.e., peak times of day, week, season), possible substitute locations, characteristics of substitute sites relative to the primary site, and likely levels of activities.

E.7.b.3 Availability of Use Level Data

The availability of use level data for those activities potentially affected by the incident should be made during the Preassessment Phase. Possible data sources could include:

- Local knowledgeable officials
- Revenue generating agencies
- Enforcement officials
- Local recreation businesses
- Available databases on recreation activities

E.7.b.4 Assessment on Potential Injury Levels

Determination of the potential injury levels should be made during the Preassessment Phase. This determination can be made by combining the information gathered from the previous three steps of data collection. If the extent of injury, or resultant effects, cannot be determined, preassessment for these activities should cease; otherwise preassessment should continue to calculate preliminary estimates of damages.

E.8 Data Collection to Assess Navigational Effects

E.8.a Objectives

One of the potential services provided by surface water is the provision of navigational access. Preassessment of navigation patterns is conducted to determine whether the discharged oil and its degradation by-products have measurable effects on the loss of surface water for navigation. Injury to surface water resources can be measured, in part, through the loss of navigational access. This loss of access can be measured by comparing vessel movements predicted to have occurred in the absence of the incident with the actual vessel traffic patterns during the incident. Preassessment activities should include the determination of port closures and potential levels of shipping activity.

E.8.b Methods

During the preassessment activities, tasks should include the determination of access closures and of typical navigation access levels. Information regarding closures of navigation access can be obtained from the USCG “Captain of the Port Notices.” Closures typically vary by time of day and location during an incident and thus daily if not hourly closures should be documented. Information regarding typical shipping patterns may be obtained through either of two primary sources, depending on the amount of shipping activity. In the case that a Vessel Traffic Service (VTS) is present in the port, information relevant to this preassessment activity can be obtained from this system. Information available from the VTS includes date and time of entry into the VTS reporting area, vessel name, next and previous port of call, vessel length, vessel draft, vessel beam, and vessel type (e.g. tanker, container ship, etc.). In addition, information regarding the location and time of vessels anchored outside the port, as a result of the closure, should be obtained.

In the event that no VTS is present in the port, the information necessary for surface water effects through lost navigational access can be obtained from vessel logs. Vessel logs are available from the shipping agents of the various vessels. The local Harbor Pilots Association can list those ships requiring a harbor pilot to enter the port and thus can identify the appropriate shipping agent. Information to be obtained from the vessel logs is similar to that obtained from the VTS reports.

Preassessment activities should include the determination of port closures and potential levels of shipping activity. If the extent of navigation access effects cannot be determined, preassessment for this activity should cease, otherwise preassessment should continue with estimating damages.

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